

EFFECT OF PROBIOTICS FERMENTATION ON ANTIOXIDANT

PROPERTY OF OAT MILK

**FOR PARTIAL FULFILLMENT OF
THE MASTER OF SCIENCE DEGREE IN LIFE SCIENCE**

2012-2014



Submitted by

PRIYA NAYAK

ROLL NO – 412LS2046

DEPARTMENT OF LIFE SCIENCE

Supervisor

Dr. R. JAYABALAN

ASSISTANT PROFESSOR

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA-769008, ODISHA



राष्ट्रीय प्रौद्योगिकी संस्थान
NATIONAL INSTITUTE OF TECHNOLOGY
राउरकेला ROURKELA - 769008, ओडिशा ODISHA



Dr. R. Jayabalan
Assistant Professor
Department of Life Science

Date: 11.05.14

CERTIFICATE

This is to certify that the thesis entitled “**DEVELOPMENT OF OAT BASED PROBIOTIC DRINK**” which is being submitted by **Ms. Priya Nayak**, Roll No. **412LS2046**, for the degree of Masters of Science in Life Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

R. Jayabalan
R. Jayabalan.

DECLARATION

I hereby declare that the thesis entitled "**Development of oat based probiotic drink**" submitted to the Department of LIFE SCIENCE, National Institute of Technology, Rourkela for the partial fulfilment of the Master of Science in Life Science is a faithful record of original research work carried out by me under the guidance and supervision of Dr. R. Jayabalan, Department of Life Science, NIT, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date: 11/05/2014

Place: Rourkela

Priya Nayak

Priya Nayak

(Roll No. 412LS2046)

ACKNOWLEDGEMENTS

I express my deep sense of gratitude and reverence to my guide, Dr. R. Jayabalan, Assistant Professor, Department of Life Science, NIT Rourkela, for his valuable guidance and supervision throughout this project.

I am extremely grateful and indebted to Dr.Samir Kumar Patra, Dr.Sujit Kumar Bhutia, Dr.Surajit Das, Dr.BibekanandMallick and Dr.SumanJha for their inspiring suggestions without which it would have been difficult to carry out this work.

I am highly obliged to MR. Ajay Dethose, Miss Indira Dash, Miss. MoumitaSahoo, and Mr.Eldin Johnson, Food microbiology and Bioprocess Laboratory Ph.D. Scholars, Department of Life Science, NIT Rourkela, for their constant help and guidance.

I take the pleasure to acknowledge the constant help and support of my friends, blessings of my parents without which this project would not have been successfully completed .

LIST OF FIGURES:

1. Oat seeds
2. Structure of β glucan
3. DPPH activity in presence of an antioxidant

LIST OF TABLES:

1. Foods containing prebiotics
2. Nutritional value of oats
3. Composition of MRS Broth
4. Effect of fermentation on pH of oat milk
5. Result of DPPH Radical Scavenging Activity

CONTENTS

1. INTRODUCTION

2. LITERATURE REVIEW

3. OBJECTIVES

4. MATERIALS AND METHODS

4.1. COLLECTION OF PROBIOTIC STRAINS

4.2. PREPARATION OF OAT MILK

4.3. FERMENTATION OF OAT MILK

4.4. DETERMINATION OF pH

4.5. ANALYSIS OF ANTIOXIDANT PROPERTY BY DPPH ASSAY

5. RESULT AND DISCUSSION

6. CONCLUSION

7. REFERENCES

ABSTRACT

The live microorganisms which provide health benefits to our body by maintaining and enhancing the intestinal microbial balance are called probiotics. They are considered to be very useful for its effectiveness against a range of gastrointestinal diseases and disorders. A prebiotic is defined as a non digestible food ingredient that beneficially affects the host body by selectively stimulating the activity or growth of one or a limited number of bacteria in the colon and thus improves host health. A synbiotic is a combination of probiotics and prebiotics that beneficially affects the host by improving the survival of live microbial dietary supplements in the gastrointestinal tract. They stimulate the growth of health promoting bacteria. The microbiota management of probiotics, prebiotics and synbiotics have been developed and commercialized over the past few decades with the expressed need of increasing numbers of bifidobacteria and lactobacilli within the gastrointestinal tract. In the present work the antioxidant property of the synbiotic product made by fermentation of different probiotic strains and oats were studied. The prebiotic source oats was fermented with different strains of lactic acid bacteria to obtain a drink, combining the health benefits of probiotic culture with oat prebiotic beta glucan having antioxidant property. The antioxidant activity of the fermented oat product was studied by DPPH radical scavenging activity.

Key Words: probiotics, prebiotics, synbiotic, fermentation, lactic acid bacteria

1. INTRODUCTION

Demand of consumers in the area of food production have altered considerably in the last few years. Consumers trust that foods are the source which directly contributes to their health(Mollet et al., 2002). In today's scenario foods are aimed not only to solve the hurdle of hunger and to facilitate various necessary nutrients but also to prevent different types of nutrition related diseases and to improve physical and mental wellbeing(Tkachi et al., 2008). World health Organization and the Food and Agricultural Organization suggested that different dietary habits along with lifestyle comprise major risk factors which are related to the development of cancer, coronary heart diseases ,diabetes ,obesity osteoporosis and periodontal diseases(WHO 2003). In this view the role played by functional foods is outstanding. There is an increasing demand on such foods which is due to the enormous increase in the expenses of health care, rapid increase in life expectancy and the wish of older people for better quality of their later years(Roberfroid et al., 2007). A widely accepted definition of a functional food is any modified and qualified food that can facilitate diverse health benefits apart from the nutrients it contains. These healthy foods constitute various products consisting of reduced fat, sugar, or salt enriched with vitamins, minerals, phytochemicals, probiotic bacteria and bioactive peptides. Functional food products belong to a new variety of food that render targeted betterment in the physiological functions of the body.

1.1 THE SYNBIOTIC APPROACH FOR FORMATION OF FUNCTIONAL FOOD

Synbiotic is a novel approach in which probiotics are combined with prebiotics in such a way that they provide benefits to the host. In the gastrointestinal tract they enhance the growth and activity of microbes and their dietary supplements.(Tuohy et al., 2003). They do so by accelerating the activity and by triggering the metabolic processes of a number of bacteria which

are helpful for promoting the health.. In this way they help in enhancing and providing welfare to the host(Gibson et al., 1995). Objective of synbiotics is to accelerate the development and action of probiotics. To study the effectiveness of synbiotics various studies were carried out in humans. A fermented milk product consisting of yoghurt starter strains and *L.acidophilus* with fructooligosaccharides resulting in decrease of total serum cholesterol level was detected (Schaafsma G. et al., 1998). A number of human feeding studies have also shown that probiotics, prebiotics and synbiotics can regulate the human gut microbiota to enhance the number and action of bifidobacteria and lactobacilli. Intake of food items containing the live bacteria is the conventional way to raise the number of beneficial bacteria in the intestinal tract and the methods are implemented today also.

Now a days a combination of probiotics and prebiotics resulting in the formation of synbiotic product is a new approach and initiative in the exploitation of functional food .Various studies have shown that these functional foods are very useful in the diminution of various types of ailments and in the improvement of the gut microbiota.

The aim of the present work was to develop a synbiotic functional drink having antioxidant properties from oats by whole-grain oat substrates and potential probiotics. This was achieved by fermenting the oat milk with various probiotic strains.

2. REVIEWOF LITERATURE

2.1.FOOD

Food is any substance which when ingested render nutritional support to our body. Quality is the characteristic attribute of food that is acceptable by the consumers. This includes external factors such as appearance (size, shape, colour, gloss, and consistency), texture and flavour; factors such as federal grade standards and internal (chemical, physical and microbial). Currently foods are considered not only on the basis of taste and nutritional demands, but also in terms of their ability to cater to particular health benefits beyond their basic nutritional value. Presently the food aimed for enhancing the balance and activities of the intestinal micro flora are rendering the largest segment of the functional food market (Saarela et al., 2002). Intake of food items containing a particular class of live bacteria is the ancient method to accelerate the working of advantageous bacteria in the intestinal tract. Such live bacteria are called as probiotics. They have been predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*. These two groups of bacteria have been broadly analysed and established as the valuable native inhabitants of the gastrointestinal tract (Salminen et al., 1998, Capela et al., 2006). Different microorganisms especially species of *Lactobacillus* and *Streptococcus* have been used conventionally in fermented dairy products to upgrade human health as well as functionality and flavour of the food.

2.2. PROBIOTICS

Probiotics are a group of live microorganisms which reside in the intestine of our body and provide health benefits to the host (humans or animals) considerably through maintaining and amending the microbial balance between the beneficial and harmful microorganisms of the intestine (Fuller et al., 1989). The word probiotic is derived from the Greek word which means for “life”. Probiotics were first defined by Kollath in 1953 to signify all organic and inorganic food complexes and compared them with harmful antibiotics. Lilly and Stillwell (1965) defined

probiotics as microorganisms which promote the growth of other microorganisms. Veld (1992) have defined probiotics as “mono or mixed cultures of live microorganisms which when employed to animal or human, give beneficial effects to the host by improving the properties of indigenous micro flora”. Probiotics may beneficially modify the normal gut flora when they are present in yogurt and other fermented food products (Metchnikoff, 1907). Probiotics have also been defined by the European Union Expert Group on functional food in Europe to be “feasible formulations in foods or dietary supplements which ameliorate the health of animals and humans”. More recently, probiotics are mentioned as “live microorganisms which when administered in adequate amounts render health benefits to the host.

2.3. HISTORICAL PERSPECTIVES OF PROBIOTICS

Escherich depicted the microbiota of the infant gastrointestinal tract (GIT) and suggested their benefits of settlement in digestion. At the same time Doderlin postulated the association of vaginal bacteria in inhibiting the development of pathogenic bacteria by producing lactic acid (Goktepe et al., 2006). Moro in 1900 and Beijerinck in 1901 described the beneficial association of Lactic acid bacteria (LAB) with human (Goktepe et al., 2006). The longevity of Caucasians was related to the high consumption of fermented milk products elucidated in his best-selling book “the prolongation of life” (Metchnikoff, 1907). LAB (Lactic Acid Bacteria) constitute a group of Gram positive, non-respiring, non sporulating cocci or rods, which produce lactic acid as a major metabolic end product during the fermentation of carbohydrates (Salminen et al., 1998). Bifidobacteria are another group of lactic acid producing bacteria but they are phylogenetically different. Bifidobacteria are found in the faeces of breast fed infants (Goktepe et al., 2006).

2.4. PROBIOTIC BACTERIA

Fermented dairy products are being manufactured traditionally from the strains of Lactic acid bacteria (LAB) such as *Lactobacillus*, *Bifidobacterium*, *Eubacterium* and *Streptococcus*. Also these bacteria are desirable members of intestinal microbiota. Factors that have been used for selecting probiotics are lack of pathogenicity, tolerance to gastrointestinal conditions (acid and bile), adherence to the gastrointestinal mucosa and competitive expulsion of the pathogens. Probiotic bacteria have been rapidly used in yogurts and fermented dairy products during the past years because of their health benefits. The most commonly used among them are lactobacilli such as *Lactobacillus acidophilus* and *Bifidobacteria* referred to as 'bifidus' (Daly and Davis, 1998). A lot of scientific evidence is present for supporting the fact that a healthy gut micro flora can protect against various gastrointestinal diseases like gastrointestinal infections, inflammatory bowel diseases and even cancer (Haenel and Bendig, 1975; Salminen et al., 1998a). The application of probiotic bacterial culture accelerates the growth of preferred microorganisms beneficial for our body, helps in eliminating potentially harmful bacteria and reinforces the natural defence mechanisms of our body. Today sufficient facts are there to support on the positive effects of probiotics on human health. A probiotic bacterium must execute following criteria before benefiting human health:

1. It must have effective technological properties so that it can be integrated into the food products without losing their functionality and viability and also they must not produce unpleasant flavours or texture.
2. It must survive passage through the upper gastrointestinal tract and must be alive at its site of action.
3. It must have the capacity to work in the environment of the gut.

2.5. PROBIOTIC STRAINS SELECTION: IMPORTANT ASPECTS

The basis for selecting probiotics includes various factors like safety, functionality and technology. An aspect related to safety of probiotics involves the following specifications (Lee and Salminen, 1995; Donohue and Salminen, 1996):

- Strains for human consumption should be preferably of human
- They should be non-pathogenic.
- They should not be associated with diseases like infective endocarditis or GI disorders.
- They should not carry transmissible antibiotic resistance genes.

The functional requirement of probiotics should be accomplished by adapting various in vitro methods. Functionality of probiotics include following aspects:

- They should be acid tolerance and tolerance to human gastric juices.
- Bile tolerance ability should be present.
- They should exhibit immunostimulation without proinflammatory effect (Isolauri et al., 1999).
- Antagonistic activity against various pathogens should be present.
- They should have the ability to adhere to epithelial surfaces and to persist in the human gastrointestinal tract (Salminen et al., 1996a).
- Antimutagenic and anticarcinogenic properties (Fernandes et al., 1987; Lidbeck et al., 1992a).

Several technological aspects (Young, 1998) have to be considered for probiotic selection which includes the following:

- Good sensory properties of the strain.
- Phage resistance, Viability during processing.
- Stability in the product and during storage

2.6. ACID AND BILE TOLERANCE OF PROBIOTICS

Some important factors like tolerance to bile and low gastrointestinal pH are the important factors for implementing probiotics in food. There are many bacteria which do not resist low pH values. The severe acidic conditions of the stomach render adverse effect on the bacteria. Therefore it is advisable that the microbial cultures to be used as probiotics should be screened for their resistance to acidity and bile tolerance (Conway et al., 1987). As soon as the bacteria reach at the intestinal tract, their ability to survive depends largely on their resistance to bile (Gilliland et al. 1984). Bile which enters into the duodenal section of the small intestine has the potential to reduce the survival of bacteria. This is because of the fact that cell membrane of bacteria consists of lipids and fatty acids which are susceptible to be destroyed by bile salts. Hence the success of a probiotic strain depends largely on the selected strain having bile and pH resistant attributes.

2.7. FOOD WITH PROBIOTICS

Food containing natural probiotics includes:

- **Yogurt:** It is a fermented milk product produced by bacterial fermentation of food. It is considered to be a good probiotic food.
- **Kefir:** Kefir refers to a drink made with kefir grains by fermenting the milk.
- **Cheese:** Cheese is a food formed by coagulation of milk protein casein.
- **Kimchi:** It refers to a traditional Korean dish obtained by fermentation of vegetables.
- **Sauerkraut:** They are finely cut cabbages fermented by lactic acid bacteria.
- **Miso:** Miso refers to a traditional Japanese seasoning which is obtained by fermentation of soybeans.
- **Tempeh:** It is also conventional soy product of Indonesia made by natural culturing and controlled fermentation process.

There are also products available which are probiotic fortified like juices, chocolate, flour and cereals.

2.8. FUNCTIONALITY OF PROBIOTICS

2.8.1. Probiotics maintaining microbial balance:

The large numbers of lactobacilli overcrowds and counteract many pathogenic bacteria in the intestine of human (De Vuyst and Vandamme , 1994 a, 1994 b). Probiotics maintain a balance between useful and harmful bacteria in the micro flora of the GI Tract (Parker, 1974; Fuller, 1989).

2.8.2. Probiotics in modulating immune system

Probiotics stimulates and maintains some specific immune responses to antigens and are considered as an important constituent of the intestine's defence mechanism (Tuohy et al., 2003). There are specific bacteria in the gastrointestinal tract that can liberate low molecular weight peptides that stimulates the immune system (Isolauri et al., 2001).

2.8.3. Probiotics in combating diarrhoea

Different probiotic strains have antidiarrheal capabilities. *Lactobacillus rhamnosus* found to decrease the duration of diarrhoea by 50 % (Isolauri et al. 1999). *Clostridium difficile* associated with diarrhoea is very severe that requires prolonged treatment with antibiotics but these treatments are not always successful. A double blind, placebo controlled trial was done in which the ability of *L. plantarum* 229v to prevent *Clostridium difficile* was studied (Wullt et al., 2003). It was found that *L. Plantarum* reduced colonisation with *C. difficile* (Klarin et al., 2008).

2.8.4. Probiotics in treatment of inflammatory bowel disease (IBD) and colorectal cancer (CRC)

Probiotics are helpful in the treatment of IBD and CRC. IBD includes a group of disorders and chronic or recurrent mucosal inflammations are its characteristics. Administration of probiotics by modulating gut microbiota or by regulating inflammatory response can help in getting relief from IBD symptoms (K.M. Tuohy et al., 2003).

Cases of CRC are found to be sporadic and accelerate with age. Diet associated with gut flora along with decreased protection from the ageing microbiota has a great contribution. Probiotics have been studied for their potential to regulate biomarkers of CRC (K.M. Tuohy et al., 2003).

2.9. CONSUMPTION OF LIVE LACTIC ACID BACTERIA

Intake of live LAB from foods fermented by lactic acid has become a regular part of food consumption in humans and it has been continuing for a long time. In this way humans have ingested large numbers of LAB. According to the original concept of probiotics, balance between beneficial and harmful bacteria in the micro flora of the GI tract can be positively altered by taking the right type of live microorganisms (Parker 1974).

2.10. THE HIERARCHY

2.10.1. THE LACTIC ACID BACTERIA

LAB can be defined as microorganisms performing the conversion of carbohydrates to carboxylic acids in traditional terms. Food microbiologists used the term earlier, and in 1919, the Danish bacteriologist Orla Jenson defined key characteristics of LAB as the true lactic acid bacteria which establish a large natural group of non-motile, non-spore formers, gram positive cocci and rods that at fermentation of sugar result in production of lactic acid. LABs are gram-

positive, non-spore forming, catalase-negative microorganisms that do not contain cytochromes and are anaerobic but aero tolerant. They are also found to be fastidious, acid-tolerant, and strictly fermentative (either homo- or hetero); the major end product formed in sugar fermentation is lactic acid (Axelsson, 1998). From meta-analyses of published clinical trials, it has been shown that various kinds of lactic acid bacteria can be used to provide protection against antibiotic-associated diarrhoea (D'Souza et al., 2002). They can also shorten the duration of acute diarrhoeal illness occurring in children (Huang et al., 2002). The common lactic acid bacteria are *L.acidophilus*, *L.fermentum*, *L.plantarum* and *L.bulgaricus*.

2.11. LAB AS STARTER CULTURE FOR FERMENTATION

The yield of fermented food is largely based on the use of starter cultures and LAB play a very crucial role in rapid acidification. LAB helps in producing fermented food by accelerating and steering its fermentation process. The LAB plays a central role in these processes and constitutes a very long and safe history of application and consumption in the production of fermented foods and beverages (Caplic and Fitzgerald, 1999). They rapidly acidify the raw materials through the production of organic acids, mainly lactic acid. Also production of acetic acid, ethanol, aroma compounds and various enzymes by LAB is of great importance. LAB in this manner enhances shelf life and microbial safety; improve the texture and helps in producing pleasant sensory profile of the end product.

2.12. PREBIOTICS

Non digestible food ingredients which have beneficial impacts on the host by selectively stimulating the growth and activity of one or limited number of bacteria in the colon can be called as prebiotics. In this way they help in improving human health (Gibson et al., 1995). The lactobacilli and bifidobacteria are the Bacteria genera which have been selected for stimulation.

2.13. PREBIOTICS IN MODULATING THE GUT MICROBIOTA

Prebiotics are commercially available as fructooligosaccharides (FOS) (4-20 g / day), inulin (20 g / day), lactulose and galactooligosaccharides. These oligosaccharides have the ability to change the gut microbiota for a more beneficial composition with increase in the number of bifidobacteria. It has shown reproducibility in human feeding studies upon both traditional microbiology and direct molecular analysis (K.M. Tuohy et al., 2000). Although there is no daily recommended dose of prebiotics, doses of 4-20 g/day have been found to be effective. Prebiotics having doses higher than 20g/day might cause some side effects like increased flatulence and abdominal bloating. However, prebiotics are found to have a very few side effects at lower doses and are present in bananas, onions and artichokes which have been shown good safety records. Many other potential probiotics are currently under investigation which includes lactitol, xylooligosaccharides and gentiooligosaccharides (K.M. Tuohy et al 2000).

2.14. ROLE OF PREBIOTICS IN INFANT HEALTH AND NUTRITION

Infants fed bovine milk is different from the microbiota of breast fed infants. It is found that the gut microbiota of breast fed infants is predominated by bifidobacteria and infants fed bovine milk constitute a higher number of *Bacteroides spp.*, *Clostridium spp.*, and *Enterobacteriaceae* (Harmsen et al., 2000). The dominance of bifidobacteria in breast fed infants is correlated with lower intestinal infection risk. It is also thought that human milk oligosaccharides might also impart natural defence against infection by promoting a proliferation of intestinal bifidobacteria and lactobacilli (Vanddenplas et al., 2001).

The composition and structure of human milk oligosaccharides cannot be formed by the food industry, therefore prebiotics are considered to fortify in infant formulas (K.M. Tuohy et al., 2000).

2.15. PREBIOTICS AND COLON CANCER

Inulin, FOS and lactulose have positive effect on biomarkers of colorectal cancer (CRC) (K.M. Tuohy et al., 2000). They decrease the working of microbial enzymes involved in the production of various toxins and carcinogens as well as alleviation in concentration of these metabolites in faeces (Rowland et al., 2000). Lactulose can directly provide protection against DNA damage in animal models challenged with active carcinogens (Rowland et al., 1996). It was investigated that inulin and FOS lessened the number and size of precancerous lesions and tumour incidence in carcinogen treated rats (Pool-Zobel et al., 1993). From these studies it was concluded that prebiotics might have the potential to reduce the risk of CRC and might even alter CRC advancement (Pool-Zobel et al., 1993). The process through which prebiotics fight against CRC is under study, and it is thought that probably it involves changes in gut microbiota which includes change in bacterial numbers and activity.

2.16. PREBIOTICS AND INFLAMMATORY BOWEL DISEASE (IBD)

Butyrate asserts remission in IBD patients by enhancing mucosal cell proliferation and increasing the process of healing in various human studies and animal models. The application of dietary fibres and prebiotics has been studied and found out as a means of stimulating butyrate production in the colon of UC patients. Germinated barley food contains glutamine rich proteins and hemicellulose rich fibres which have been found to reduce the symptomatology in both animal models of UC and patients with UC (Bamba et al., 2002).

2.17. PREBIOTICS AND HUMAN METABOLISM

Prebiotics alter the serum triglyceride levels and cholesterol. Inulin and FOS have been associated with an improvement of mineral absorption in the large bowel (Roberfroid et al., 2000). A placebo controlled study was carried out using a stable isotope of calcium in which FOS was shown to meliorate calcium intake in women during late menopause phase (Tahiri et al., 2003). A significant improvement in calcium absorption was seen in adolescent girls taking

drink fortified with FOS and inulin. Magnesium absorption was also increased in humans and animals consuming prebiotics(Coudray et al., 2003).

These are the following food containing different percentage of prebiotics (Frank et al., 2010)(Table 1).

Table 1: foods containing prebiotics

Food	% of prebiotic
Raw chicory root	64.6 % prebiotic fibre by weight
Raw Dandelion greens	24.3% prebiotic fiber by weight
Raw Garlic	17.5% prebiotics by weight
Raw Leek	11.7% prebiotic fiber by weight
Raw onion	8.6 % prebiotics by weight
Cooked onions	5% prebiotic fiber by weight
Raw Asparagus	5% prebiotics by weight
Raw Wheat bran	5% prebiotic fiber by weight
Wheat flour, baked	4.8% prebiotics by weight
Raw banana	1 % prebiotic by weight

2.18. OATS AS PREBIOTIC

The common oat (*Avena sativa*) is a species of cereal grain for its seed (fig 1). While oats are consumed as oatmeal and rolled oats by humans, one of most common uses is as livestock.



Fig 1: Oat seeds

Scientific classification:

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Monocots
Order	Poales
Family	Poaceae
Genus	<i>Avena</i>
Species	<i>sativa</i>

2.19. CULTIVATION

Oats are grown in temperate areas. They need a lower summer heat and greater tolerance to rain than other cereals like rye, wheat or barley, so are particularly significant in areas with cool wet summers such as North West Europe.

Oats are considered as an annual plant and can be planted either in autumn season or in the spring. Known as “jau” in local areas, oats are grown on the foothills of Himalayas, such as in the state of Himachal Pradesh.

2.20. SOLUBLE FIBRE

Oats contain more soluble fibres than any other grain which results in slower digestion and an extended sensation of fullness. β glucan which is one type of soluble

fibre has been proven to help lower cholesterol. Consumption of oats results in lowering cholesterol and decreasing the risk of heart diseases.

A class of polysaccharides known as β D glucan comprise the soluble fibre in whole oats. β D Glucan, also called as beta glucan belongs of a class of indigestible polysaccharides. In oats, barley and other cereals they are present primarily in the endosperm cell wall. β glucan is a viscous polysaccharide made up of units of monosaccharide D glucose. Oat β Glucan is composed of mixed linkage polysaccharides. This implies that the D-glucose or D glucopyranosyl units are either - β 1, 3 linkages or β - 1, 4 linkages. This type of β glucan is called as a mixed linkage (fig 2).

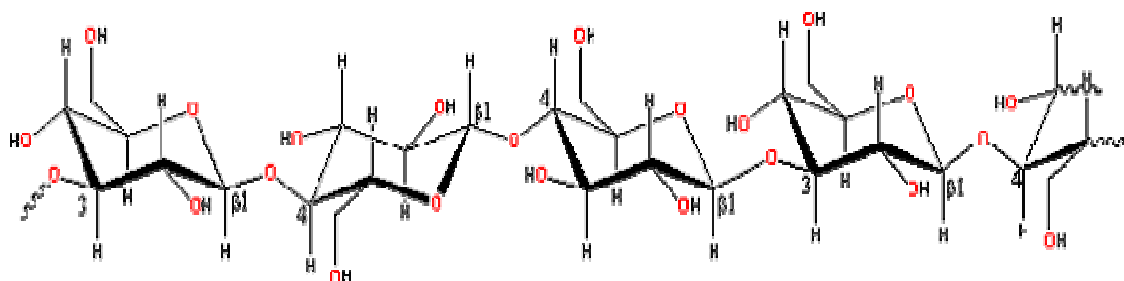


fig 2 : structure of β glucan

The percentage of B glucan in various oats products are:

Oat bran-greater than 5.5% and up to 23.0%

Rolled oats-about 4 %

Whole oat flour-4%

Oats after corn has the highest lipid content(Table 2).

Table2: Nutritional value of oats

Nutritional value per 100 g

Energy	1,628 kJ
Carbohydrates	66.3g
Dietary fibres	10.6g
Fat	6.9g
Protein	16g
Thiamine	0.763g
Riboflavin	0.139g
Niacin	0.961g
Pantothenic acid	1.349g
Folate	56µg
Calcium	54mg
Iron	5 mg
Magnesium	177mg
Phosphorus	523 mg
Potassium	429 mg
Zinc	4 mg
β glucan	4g

2.21. HEALTH BENEFITS OF OATS

2.21.1. Blood cholesterol and oats

Oats are hypocholesterolemic and result in lowering total and LDL cholesterol by 2 to 23 %. They do not reduce cholesterol level by displacing fats and cholesterol intake and also do not affect HDL cholesterol levels (Van Horn et al., 1988).

2.21.2. Reduction in diabetes

Oats contains soluble fibres and when they are incorporated into a low glycaemic diet, they improve insulinemic responses (Brand et al., 1991). Oats are found to reduce blood glucose and insulin levels.

2.21.3. Obesity and oats

Weight loss studies were conducted with oats and it was observed that more weight loss was observed when oat bran biscuits were used in daily diet (Krotkiewski et al. 1985). In addition to this, subjects who were given oat based soup as the main meal twice a day lost have significant weight loss (Rytter et al., 1996).

2.21.4. Controlling blood pressure

It was studied from animal epidemiological and clinical studies that soluble fibres are effective in lowering blood pressure. It was observed in a study that Oat bran reduce increased blood pressure in rats with high sucrose diets (Gondal et al., 1996).

2.22. DEVELOPMENT OF OAT BASED PROBIOTIC DRINK

Continuous exploitation of new functional food has become the aim of science and industry due to the increase in consumer's consciousness related to health and function of foods for enhancing the attribute of life (Bland and Medcalf et al., 2000). Due to different types of benefits, oats has become renowned as part of a healthy diet and new oat products are emerging at the functional market (Brand et al., 1991)

A large proportion of probiotics found in the Markets worldwide are milk based. Not much attempts were made for developing probiotic foods using other fermented substrates like cereals. Recently they have been concentrated for their use as raw materials in the synthesis of new fermented functional foods due to their wide distribution and important nutritive value. The major sources of β glucan are found to be oats and barley and recognised as the major functional constituent of cereal fibres. Their hypocholesterolaemic effect have been studied, and it was found that they lead to 20-30% alleviation in cholesterol level and decrease in cardiovascular disease risk (Stark and Madar, 1994). Oats and barley contain highest β glucan content among cereals (Manthey et al., 1999).

In 1997, the products made of whole oat grains or oat fibre consisting of a minimum 0.75 g β glucan has been declared as functional by the FDA of US. The oat products have low glycaemic index. This characteristic is very important for diabetes. Ingestion of food containing β glucan is found to alter the level of fat emulsion in gastro intestinal tract and slows down lipase activity (Anderson and Bridges, 1993). Low molecular fatty acids produced during fermentation of glucan in the colon shows its potential anticarcinogenic effect (Oku, 1994). Also β glucan is reckoned as a prebiotics which accelerates the growth of various useful residential colon microorganisms like bifidobacteria.

Oats is a suitable substrate for fermentation with LAB after suitable processing (Marklinder and Lonner 1992). Oats bases treated enzymatically have been produced (Martensson et al., 2002). They are used as substrates with dairy starter cultures for fermentation of lactic acid (Martensson et al., 2002). Therefore a great interest for the consumption of products based on oats is increasing that contain both soluble and insoluble fibres and have positive effects on blood cholesterol levels (Wood, 1991).

Fermentation processes with oat bases (Adavena ;Ceba Foods AB,Lund,Sweden)enriched in dietary fibres can be used to develop the interest in oats as new material for the synthesis of new functional food products. Therefore the addition of intestinal lactobacillus spp. and bifidobacterium spp. to fermented food products is being applied with lot of interest in the last few decades (Vinderola, Bailo and Reinheimer ,2000).

2.23. ANTIOXIDANT PROPERTY OF FERMENTED OATS DRINK

Foods play a crucial role in the retrieval of body from physiological stress and other pressure. Oxidative stress produces reactive oxygen species (ROS) which are called as free radicals. Also a number of oxidation reactions are occurring in our body which results in production of free radicals. These free radicals are very reactive and contain unpaired valence electron.

Free radicals are continuously produced in different forms for different metabolic requirement. They are quenched by an efficient network of antibody in the body. When these species are generated more than the levels of antioxidant mechanism, oxidative damage of tissues and biomolecules occurs, resulting in disease conditions,especially degenerative diseases.Moreover, in somestudies thebehavior of selected probiotic strains and their antioxidant activity is described (Kullisaar et al., 2002, Annuk et al., 2003). It has been suggested that Lactobacillus may improve the antioxidant defence system of the host.

2.24. DPPH RADICAL SCAVENGING ACTIVITY

This method is applied for determining the antioxidant property of any substance.1, 1-Diphenyl-2-picryl hydrazyl (DPPH) is a stable free radical and in the above mentioned method their reduction takes place. DPPH contains an odd electron and gives maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, it donates the hydrogen atom. As a result DPPH becomes paired off and gets reduced to DPPHH. The absorbance also decreases and the radical to DPPH-H form causes decolourisation to yellow

colour based on the number of captured electrons (fig 3). More is the decolourisation, more is the reducing ability (Mohammad et al., 2009).

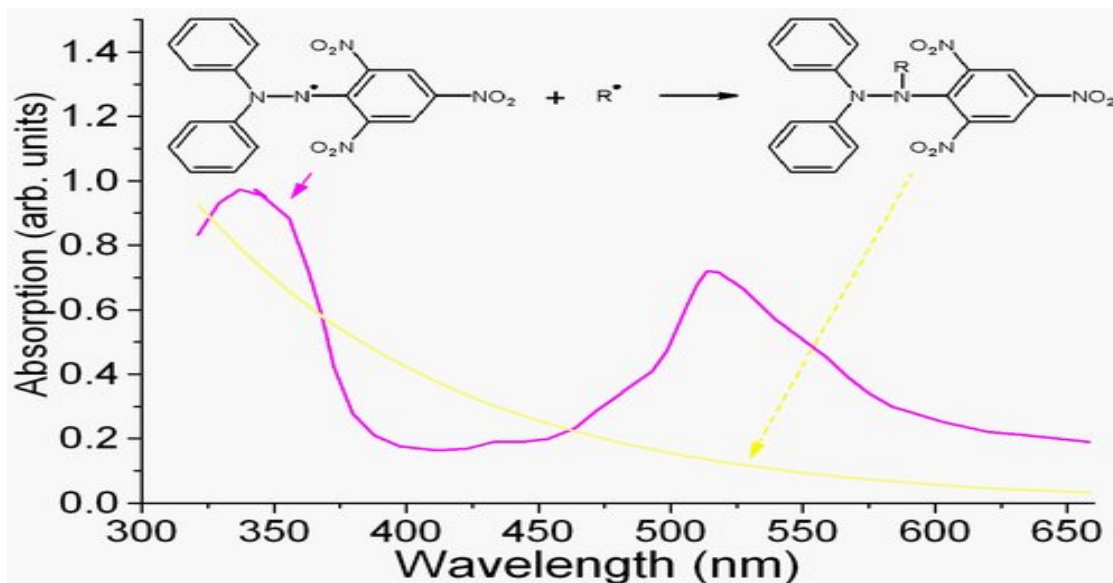


Fig 3: DPPH activity in presence of an antioxidant.

2.25. HEALTH BENEFITS OF OAT BASED PROBIOTIC DRINK

- **Antioxidant property** – Antioxidant property is an important characteristic of food and plays a crucial role as a health protecting factor. It is suggested that antioxidant can reduce the risk for various chronic diseases which include heart disease and cancer. It has been shown in the above studies that this probiotic oat drink is having antioxidant property.
- **Help in lowering cholesterol:** Various meta analyses evaluation of hypocholesterolemic effects of the compound was done and it was found that they lead to 20 – 30 % reduction in LDL cholesterol. This also results in reduction in cardiovascular disease risk (Stark and Madar , 1994)
- **Plant product:** It is a purely plant based product obtained from oats.

- **Useful for lactose intolerance people:** This drink can be considered as a dairy free alternative to milk and can be taken by lactose intolerant people. It has been found that probiotic cultures can enhance lactose digestion in lactose maldigestors.

3. OBJECTIVES:

1. To collect Lactic acid bacteria (LAB) from National Collection of Industrial Microorganisms (NCIM), Pune.
2. To prepare oat milk.
3. To ferment oat milk with LAB.
4. To determine pH change in 24 hours.
5. To study the DPPH radical scavenging activity of the fermented oat milk.
6. To prepare probiotic oat drink.

4. MATERIALS AND METHODS:

4.1. Collection of LAB:

Four probiotic strains *Lactobacillus fermentum* NCIM 2165, *Lactobacillus plantarum* NCIM 2083, *Lactobacillus bulgaricus* NCIM 2056, *Lactobacillus acidophilus* NCIM 2660 were collected from NCIM, Pune.

4.2 . Preparation of oat milk:

Steel cut oats were soaked in water for 10 minutes. Then the soaked oats and water were blended properly in a grinder in 1: 3 ratio. The liquid solution obtained was filtered by using a cotton cloth. The solution obtained was the oat milk. The pH of the solution was checked and kept in refrigerator.

4.3 . Fermentation of oat milk:

The four probiotic strains *Lactobacillus fermentum* NCIM 2165, *Lactobacillus plantarum* NCIM 2083 , *Lactobacillus bulgaricus* NCIM 2056 , *Lactobacillus acidophilus* NCIM 2660 were inoculated in MRS broth (Table 3) and kept in the incubator for 24 hours. The broth was then centrifuged for 10 minutes at 7000 RPM. The supernatant obtained was discarded and the pellets left over were washed with saline water twice. This step was followed by inoculating the pellets in oat milk. Then the oat milk was kept in incubator for 24 hours.

Table 3 : Composition of MRS Broth

Composition	Concentration (g/ l)
Enzymatic Digest of Animal Tissue	10

Beef Extract	10
Yeast Extract	5
Dextrose	20
Sodium Acetate	5
Polysorbate 80	1
Potassium Phosphate	2
Ammonium Citrate	2
Magnesium Sulfate	0.1
Manganese Sulfate	0.05

4.4 Determination of pH

pH of the fermented product was checked regularly for 0 , 6 , 12 and 24 hours.

4.5.DPPH assay for analysis of antioxidant property:

Four probiotic strains *L. acidophilus*, *L. bulgaricus*, *L. fermentum* and *L. plantarum* were inoculated in the oat milk in four separate tubes. In one tube 0.1 g / ml of vit C was taken and in another tube oat milk without fermentation was taken. 100 µl of each sample were mixed with 1 ml DPPH and 1.9 µl 50mM tris HCl and kept in dark for 30 minutes. 1 ml DPPH, 1.9 ml Tris Hcl and 100 µl water served as control. Reduction of DPPH free radicals was measured by reading absorbance at 517 nm.

The activity is given as % DPPH radical scavenging and calculated as follows: DPPH radical scavenging activity % = [(control absorbance – sample absorbance) / control absorbance] * 100

5. RESULT AND DISCUSSION

5.1 . Effect of fermentation on pH of oat milk:

The pH was checked regularly at 0,6, 12 and24 hours and it was found that initial pH was 5.5 and within 24 hours the pH got reduced to 4(Table 4).

Table 4 : Effect fermentation on pH of oat milk:

Time (in hours)	0	6	12	24
pH	5.5	5	5	4

5.2. DPPH radical scavenging activity (Table 5):

The absorbance was taken at 517 nm and the following result was found (Table 5).

Table 5 : Result of dpph radical scavenging activity

Samples	Radical scavenging activity (%)
Vit C (positive control)	68.5
Unfermented oat milk	0
<i>L. acidophilus</i>	7.93
<i>L. bulgaricus</i>	7.06
<i>L. fermentum</i>	2.06
<i>L . plantarum</i>	0.517

The highest antioxidant capacity was seen in case of *L. acidophilus* followed by *L. bulgaricus* ,
L.fermentum , *L. plantarum*.

CONCLUSION:

Oat milk without fermentation does not show any antioxidant property. Fermentation of oat milk with lactic acid bacteria increased the antioxidant activity of oat milk. Oat milk fermented with *L. acidophilus* is having the highest antioxidant property followed by *L. bulgaricus*. Oat milk fermented with *L. fermentum* and *L. plantarum* have the least antioxidant property. During fermentation, the β -glucan present in oat milk might have got converted into some simpler substances by the enzymes produced by lactic acid bacteria and in the presence of organic acids. The converted compounds may exhibit antioxidant activity.

REFERENCES:

1. Anderson, J.W., Bridges, S.R., 1993. Hypcholesterolemic effects of oat bran in humans. In: Wood, P.J. (Ed.), Oat Bran. American Association of Cereal Chemists, US, St. Paul, pp. 139–157.
2. Bland, J.S., Medcalf, D.G., 1994. Future prospects for functional foods. In: Goldberg, I. (Ed.), Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. Chapman & Hall, New York, pp. 537–551.
3. De Vuyst, L. and Vandamme, E.J. (1994a). Antimicrobial potential of lactic acid bacteria, in *Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications*, De Vuyst, L. and Vandamme, E.J., Eds., Blackie Academic & Professional, London, pp. 91-142.
4. De Vuyst, L., and Vandamme, E.J. (1994b). Lactic acid bacteria and bacteriocins: Their practical importance, in *Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications*, De Vuyst, L. and Vandamme, E.J., Eds., Blackie Academic & Professional, London, pp. 1-11.
5. Donohue, D.C., Salminen, S.J., 1996. Safety of probiotic bacteria. Asia Pacific J. Clin. Nutr. 5, 25–28.
6. Fernandes, C.F., Shahani, K.M., Amer, M.A., 1987. Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. FEMS Microbiol. Rev. 46,343–356.
7. Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365–378.
8. Goktepe, I., Juneja, V. K. and Ahmedna, M., 2006. Probiotics in food safety and human health. Boca Raton, FL: Taylor and Francis group.
9. Gibson, G.R. et al. (2000) *Prebiotics: New Developments in Functional Foods*, Chandos Publishing.

9. Isolauri, E., Salminen, S., Mattila-Sandholm, T., 1999. New functional foods in the treatment of food allergy. *Ann. Med.* 31, 299–302.
10. Klarin, B., Wult, M., Palmquist, I., Molin, G., Larsson, A. & Jeppsson, B. (2008). *Lactobacillus plantarum* 299v reduces colonisation of *Clostridium difficile* in critically ill patients treated with antibiotics. *Acta Anaesthesiologica Scandinavica* 52: 1096-1102.
11. Lee, Y.-K., Salminen, S., 1995. The coming of age of probiotics. *Trends Food Sci. Technol.* 6, 241–245.
12. Manthey, F.A., Hareland, G.A., Huseby, D.J., 1999. Soluble and insoluble dietary fiber content and composition in oat. *Cereal Chemistry* 76 (3), 417–420.
13. Marklinder, I., Lonner, C., 1992. Fermentation properties of intestinal strains of *Lactobacillus*, of a sour dough and of a yoghurt starter culture in an oat based nutritive solution. *Food Microbiology* 9, 197–205.
14. Martensson, O., Staaf, J., Duecas-Chasco, M., Irastorza, A., Oste, R., Holst, O., 2002. A fermented, ropy, non-dairy oat product based on the exopolysaccharide-producing strain *Pediococcus damnosus*. *Advances in Food Sciences* 24, 4–1
15. Mohammad A.E., Seyed M.N., Seyed F.N., Bahramian F., Bekhradnia A.R. Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. *Pak. J. Pharm. Sci.* 2010; 23(1): 29-34.
16. Mollet, B., & Rowland, I. (2002). Functional foods: at the frontier between food and pharma. *Current Opinion in Biotechnology*, 13, 483e485.
17. Oku, T., 1994. Special physiological functions of newly developed mono- and oligosaccharides. In: Goldberg, I. (Ed.), *Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals*. Chapman & Hall, New York, pp. 202–218.

18. Parker, R.B. (1974). Probiotics, the other half of the antibiotic story, *Anim. Nutr. Health* 29: 4-8.
19. Pool-Zobel, B.L., Bertram, B., Knoll, M., Lamertz, R., Neudecker, C., Schillinger, U., Schmezer, P., Holzapfel, W.H., 1993. Antigenotoxic properties of lactic acid bacteria *in vivo* in the gastrointestinal tract of rats. *Nutr. Cancer* 20, 271–281.
20. Roberfroid, M. B. (2007). Concepts and strategy of functional foodscience: the European perspective. *The American Journal of Clinical Nutrition*, 71, 1660e1664.
21. Schaafsma, G. *et al.* (1998) Effects of a milk product, fermented by *Lactobacillus acidophilus* and with fructo-oligosaccharides added, on blood lipids in male volunteers. *Eur. J. Clin. Nutr.* 52, 436–440.
22. Saarela, M., Lathenmaki, L., Crittenden, R., Salminen, S., and Mattilasandholm, T., 2002. Gut bacteria and health foods - the European perspective. *Int. J. Food Microbiol.*, 78: 99-117.
23. Salminen, S., and Wright, V. A., 1998a. Lactic acid bacteria: microbiology and functional aspects, second edition, revised and expanded. New York: Marcel Dekker, Inc.
24. Tuohy, K.M. *et al.* (2002) A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. *Microb. Ecol. Health Dis.* 14, 165–173.
25. Vinderola, C.G., Costa, G.A., Regenhardt, S., Reinheimer, J.A., 2002. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. *International Dairy Journal* 12, 579–589.
26. Wullt, M., Johansson Hagslätt, M.-L. and Odenholt, I. (2003). *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated

diarrhoea: A double-blind placebo-controlled trial. *Scandinavian Journal of Infect. Dis.* 35: 365-367.